Listing of Claims:

1-14. (Canceled)

15. (Previously presented) A method for determining the efficiency of an amplification of a target nucleic acid comprising the steps of:

(a) preparing a dilution series of the target nucleic acid;

(b) amplifying the target nucleic acid under defined reaction conditions and measuring the amplification in real-time;

(c) setting a defined signal threshold value;

(d) determining, for each dilution, the cycle number at which the signal threshold value is exceeded;

(e) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid used for the amplification as a function of the cycle number at which the signal threshold value is exceeded, wherein the non-linear continuously differentiable function from step (e) is determined with a polynomial fit of the 3^{rd}, 4^{th}, 5^{th}, 6^{th} or 7^{th} degree; and

(f) calculating the amplification efficiency from said non-linear continuously differentiable function.

16. (Previously presented) The method of claim 15, wherein the amplified nucleic acid is detected with a DNA-binding dye.

17. (Previously presented) The method of claim 16, wherein said DNA-binding dye is SYBR® Green I.

18.-22. (Canceled)

23. (Previously presented) A method for determining amplification efficiency of a nucleic acid as a function of concentration comprising:

(a) amplifying different dilutions of a nucleic acid and measuring their amplification in real-time;

(b) determining the cycle number at which amplification exceeds a threshold for each of the dilutions; and

(c) determining amplification efficiency of the nucleic acid as a function of concentration, from a non-linear, continuously differentiable function of the cycle number determined in step (b) that maps said cycle number to the logarithm of concentration of the target nucleic acid, wherein the non-linear, continuously differentiable function is determined with a polynomial fit.

24. (Previously presented) The method of claim 23, wherein said polynomial fit is of the 3^{rd}, 4^{th}, 5^{th}, 6^{th} or 7^{th} degree.

25. (Previously presented) A method for determining amplification efficiency of a nucleic acid as a function of concentration comprising:

(a) amplifying different dilutions of a nucleic acid and measuring their amplification in real-time;

(b) determining the cycle number at which amplification exceeds a threshold for each of the dilutions; and

(c) determining amplification efficiency of the nucleic acid as a function of concentration, from a non-linear, continuously differentiable function of the logarithm of concentration of nucleic acid that maps said logarithm of concentration to the cycle number determined in step (b), wherein the non-linear, continuously differentiable function is determined with a polynomial fit.

26. (Previously presented) The method of claim 25, wherein said polynomial fit is of the 3^{rd}, 4^{th}, 5^{th}, 6^{th} or 7^{th} degree.

27.-30. (Canceled)

31. (Currently Amended) A method for quantifying a target nucleic acid relative to a reference nucleic acid in a sample to be analyzed, wherein the sample to be analyzed comprises the target nucleic acid and the reference nucleic acid, comprising:

(a) amplifying different dilutions of the target nucleic acid and different dilutions of the reference nucleic acid and measuring amplification in real-time;

(b) determining the cycle number at which amplification exceeds a first threshold for each of the dilutions of step (a);

(c) generating a non-linear continuously differentiable target function, F_T, of target nucleic acid cycle number and a non-linear continuously differentiable reference function, F_R, of reference nucleic acid cycle number wherein:

F_T maps the cycle numbers determined in step (b) for the dilutions of the target nucleic acid to the logarithm of concentration of the target nucleic acid and

F_R maps the cycle numbers determined in step (b) for the dilutions of the reference nucleic acid to the logarithm of concentration of the reference nucleic acid;

(d) amplifying the target nucleic acid and the reference nucleic acid in the sample to be analysed under similar amplification conditions and measuring amplification in real-time;

(e) amplifying a calibrator sample and measuring amplification in real-time, wherein the calibrator sample comprises the target and reference nucleic acids in a known concentration ratio;

(f) determining F_T(Cp-Tar), F_R(Cp-Ref), $F_T(\text{Cp-Tar}_{cal})$ and $F_R(\text{Cp-Ref}_{cal})$, wherein:

F_T(Cp-Tar) is the value of the target function, F_T, at the cycle number (Cp-Tar) at which the amplification exceeds a second threshold for the target nucleic acid in step (d),

F_R(Cp-Ref) is the value of the reference function, F_R, at the cycle number (Cp-Ref) at which the amplification exceeds the second threshold for the reference nucleic acid in step (d),

F_T(Cp-Tar_{cal}) is the value of the target function, F_T, at the cycle number (Cp-Tar_{cal}) at which the amplification exceeds the second threshold for the target nucleic acid in step (e) and

F_R(Cp-Ref_{cal}) is the value of the reference function, F_R, at the cycle number (Cp-Ref_{cal}) at which the amplification exceeds the second threshold for the reference nucleic acid in step (e); and

(g) quantifying the amount of target nucleic acid relative to the reference nucleic acid, wherein, the relative amount is:

$$\frac{F_T(\text{Cp-Tar}) / F_R(\text{Cp-Ref})}{F_T(\text{Cp-Tar}_{cal}) / F_R(\text{Cp-Ref}_{cal})}$$

32. (Previously presented) The method of claim 31, wherein the continuously differentiable function is determined with a polynomial fit.

33. (Previously presented) The method of claim 32, wherein said polynomial fit is of the 3rd, 4th, 5th, 6th or 7th degree.

34. (Previously presented) The method of claim 31, wherein the amplified nucleic acid is detected with at least one fluorescently labeled hybridization probe.

35. (Previously presented) The method of claim 34, wherein the amplified nucleic acid is detected with FRET hybridization probes, molecular beacons, or TAQMAN® probes.

36. (Previously presented) The method of claim 31, wherein the amplified nucleic acid is detected with a DNA-binding dye.

37. (Previously presented) The method of claim 36, wherein said DNA-binding dye is SYBR® Green I.

38. (Previously presented) A method for absolute quantification of a target nucleic acid in a sample comprising the steps of:

(a) determining the amplification efficiencies of the target nucleic acid and of an internal or external standard under defined amplification conditions by:

(i) preparing a dilution series of the target nucleic acid and a dilution series of the internal or external standard;

(ii) amplifying the target nucleic acid and the internal or external standard under defined reaction conditions and measuring the amplification in real-time;

(iii) setting a defined signal threshold value;

(iv) determining, for each dilution of the target nucleic acid and for each dilution of the internal or external standard, the cycle number at which the signal threshold value is exceeded;

(v) determining a non-linear continuously differentiable function of the cycle number determined in step (iv) as a function of the logarithm of the copy number of target nucleic acid and the internal or external standard used for the amplification; and

(vi) calculating the amplification efficiency of the target nucleic acid and the internal or external standard from said non-linear continuously differentiable function;

(b) amplifying the target nucleic acid contained in the sample and the internal or external standard under said defined reaction conditions;

(c) measuring the amplification of the target nucleic acid and that of the internal or external standard in real time; and

(d) calculating the original copy number in the sample by correcting the copy number derived from step c) with the amplification efficiencies determined in step a).

39. (Previously presented) A method for quantification of a target nucleic acid in a sample relative to a reference nucleic acid comprising the steps of:

(a) determining the amplification efficiencies of the target nucleic acid and of the reference nucleic acid under defined amplification conditions by:

(i) preparing a dilution series of the target nucleic acid and a dilution series of the reference nucleic acid;

(ii) amplifying the target nucleic acid and the reference nucleic acid under defined reaction conditions and measuring the amplification in real-time;

(iii) setting a defined signal threshold value;

(iv) determining, for each dilution of the target nucleic acid and for each dilution of reference nucleic acid, the cycle number at which the signal threshold value is exceeded;

(v) determining a non-linear continuously differentiable function of the cycle number determined in step (iv) as a function of the logarithm of the copy number of target nucleic acid and the reference nucleic acid used for the amplification; and

(vi) calculating the amplification efficiency of the target nucleic acid and the reference nucleic acid from said non-linear continuously differentiable function;

(b) amplifying the target nucleic acid contained in the sample as well as the reference nucleic acid contained in the sample under said defined amplification conditions;

(c) measuring the amplification of the target nucleic acid and that of the reference nucleic acid in real time; and

(d) calculating the original ratio of target nucleic acid and reference nucleic acid in the sample by correcting the ratio derived from step c) with the amplification efficiencies determined in step a).

40. (Previously presented) The method of claim 15, wherein the amplified nucleic acid is detected with at least one fluorescently labeled hybridization probe.

41. (Previously presented) The method of claim 40, wherein the amplified nucleic acid is detected with FRET hybridization probes, molecular beacons, or TAQMAN® probes.